lac Promoter
KpnI
ApaI
DraII
XhoI
SalI HincII AccI
ClaI
HindIII
EcoRV
EcoRI
PstI
BamHI
EnHCH
NcoI
NdeI
BamHI
HindIII
EcoRI
PstI
SmaI
BamHI
SpeI
XbaI
NotI
EagI
SacII
BstXI
SacI
pBluescript KS

Fig. 1

PstI
EnHCH
BamHI
DNA sequence which
encodes signal peptide

Fig. 2

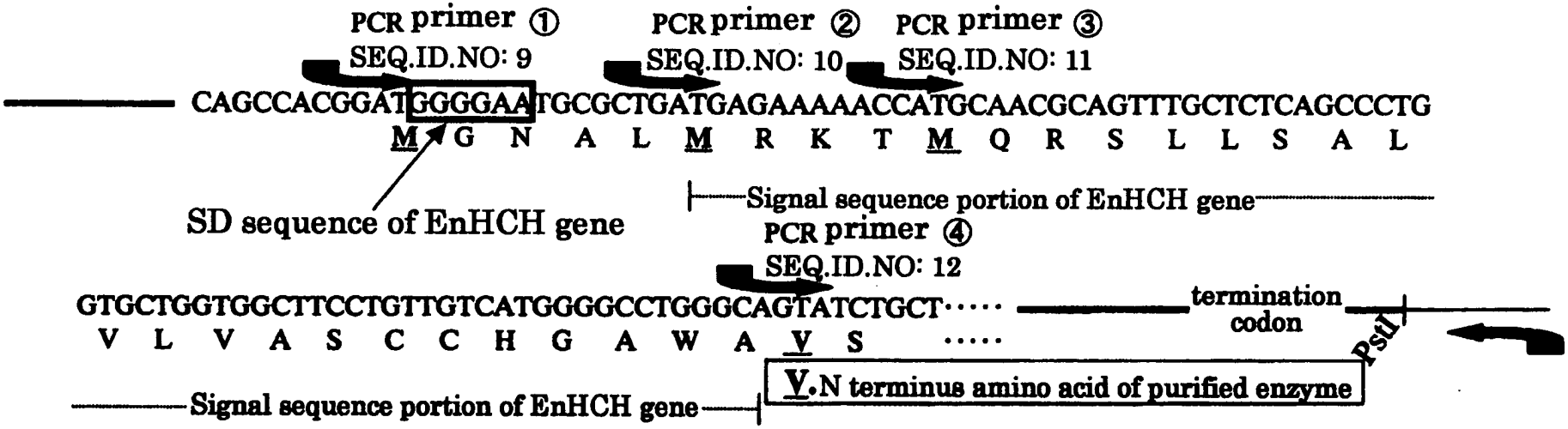
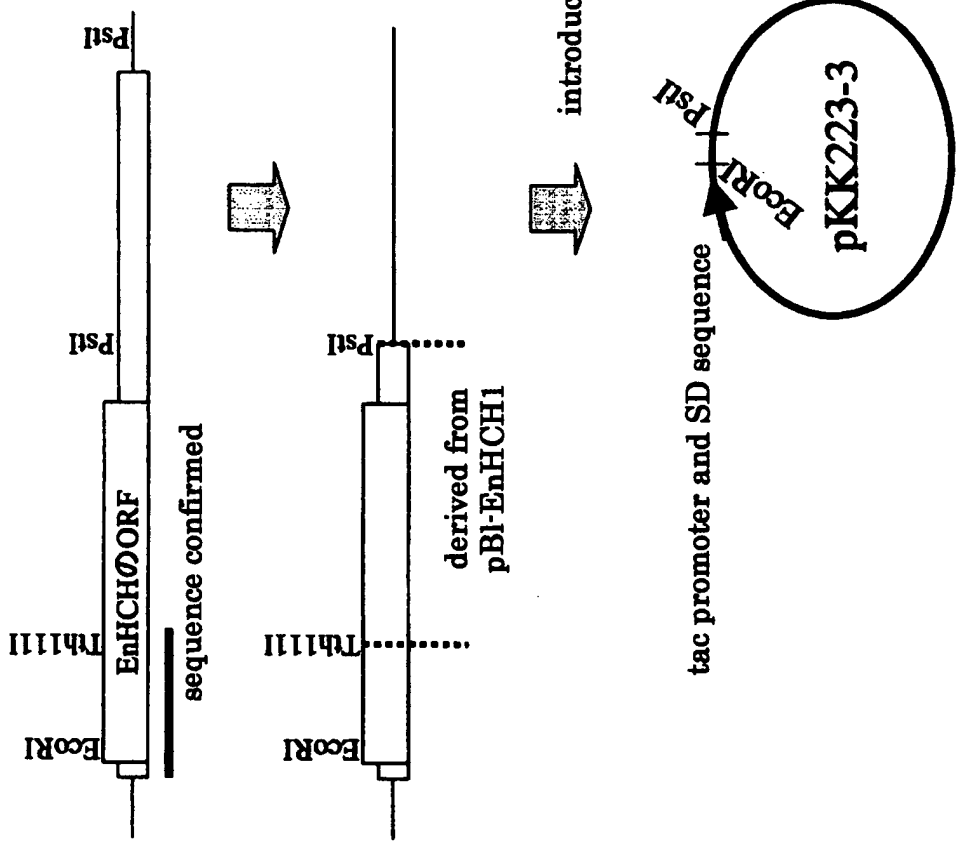

PCR primer ①
SEQ.ID.NO: 9
PCR primer ②
SEQ.ID.NO: 10
PCR primer ③
SEQ.ID.NO: 11
CAGCCACGGATGGGGAATGCGCTGATGAGAAAAACCATGCAACGCAGTTTGCTCTCAGCCCTG
M G N A L M R K T M Q R S L L S A L
SD sequence of EnHCH gene
Signal sequence portion of EnHCH gene
PCR primer ④
SEQ.ID.NO: 12
GTGCTGGTGGCTTCCTGTTGTCATGGGGCCTGGGCAGTATCTGCT·····
termination codon
V L V A S C C H G A W A V S ·····
PstI
V.N terminus amino acid of purified enzyme
Signal sequence portion of EnHCH gene

Fig.3


4 kinds of PCR products subjected to subcloning to pUC118 at blunt end and having different upstreams (PCR products of PCR primer ① to ④ and M13 primer)
exchanged with pBI-EnHCH drived between Tth111I-PstI
introduced into pKK223-3 at EcoRI-PstI
PstI
EnHCHØORF
Tth111I
EcoRI
sequence confirmed
derived from pBI-EnHCH1
tac promoter and SD sequence
pKK223-3

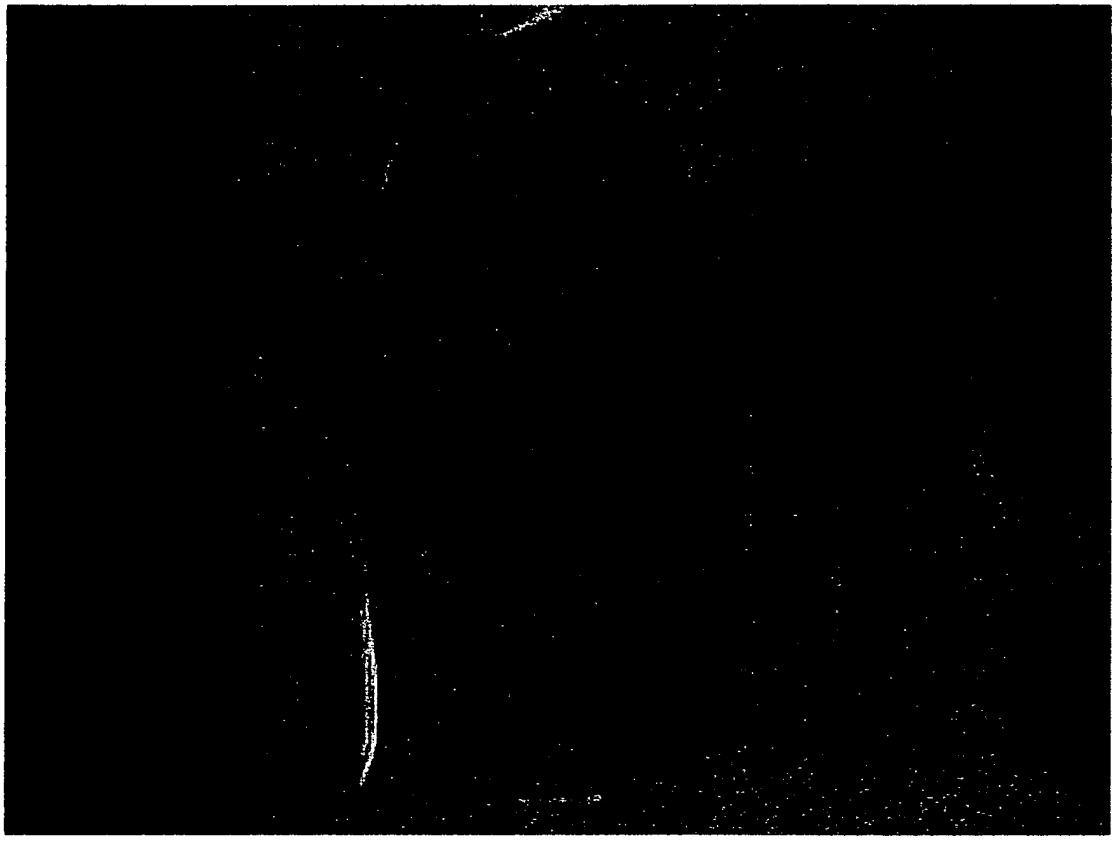



Fig.4